

IN THE UNITED STATES PATENT AND
TRADEMARK OFFICE

Provisional Patent Application



REAL TIME BIO CHIP IMAGER

This novel technology provides an extremely sensitive optical imaging system for real-time readout of the binding status of biomolecular array elements on the surface of a glass or plastic chip. The monitored area is approximately 25 mm in diameter, with a lateral resolution better than 10 microns, resulting in fully parallel, continuous real-time readout of up to 5 million sensor fields. System sensitivity to surface attachment is in the femtogram/mm² range. IOS's replacement of conventional mechanical scanning with an imaging technique offers numerous advantages, and enables the monitoring of dynamic binding processes in all sensing fields simultaneously.

APPLICATIONS

- DNA and protein chip scanning
- High throughput screening
- Ligand fishing
- Immunosensors
- Clinical diagnostics and research
- Toxicology profiling
- Binding kinetics research
- Genomics
- Proteomics

FEATURES

- Real-time
- No labels necessary
- Sensitivity: 1 fg/mm² (one DNA per square micron)
- Spatial resolution: 20 microns
- Sensor field area: 25 mm diameter

UTILITY PATENT APPLICATION

TITLE: APPARATUS INCLUDING A BIOCHIP FOR IMAGING OF
BIOLOGICAL SAMPLES AND METHOD

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REFERENCE TO RELATED APPLICATIONS

This application is a Continuation-in-Part of application Serial No. 09/614,503
filed on 7 July 2000 the content of which is incorporated by reference herein. This
10 application is based in part on Provisional Patent Application Serial No: 09/614, 503 filed
on 27 October, 2000 the content of which is incorporated by reference herein.

FIELD OF THE INVENTION

This invention relates to imaging techniques in conjunction with total internal reflection
at the boundary of an optically transparent material and more particularly to the use of
15 such techniques for detecting the presence, composition, quantity, and spatial distribution
of substances on optically transparent substrates.

This invention relates to a biochip (also referred to as gene chip, protein chip,
microarray and others) useful in applications of the ans caused the local polarization
change detected in the respective parts of the emerging light beam. U.S. Pat. No.
20 5,633,724 to Ring, et al. (1997) describes the readout of a biochemical array using the
evanescent field. This patent focuses on fluorescent assays, using the evanescent field to
excite fluorescent markers attached to the substances to be detected and analyzed. The
attachment of fluorescent markers or other molecular tags to the substances to be detected
on the surface requires an additional step in performing the measurement, which is not
25 required in the current invention. The patent further describes use of a resonant cavity to
provide on an evanescent field for exciting analytes.

The formation of an array of biologically active spots on the surface of a substrate for
identifying constituents in test material brought into contact with the array is well known.
Typically, such processes require spots of, for example, oligonucleotides, DNA clones,
30 antibodies, peptides, receptors, enzymes, inhibitors, etc. which are processed to exhibit
fluorescence, electroluminescence, current change, voltage change - - etc. for providing a

detectable signature for the presence of constituents in the material being tested.

SUMMARY OF THE INVENTION

In accordance with the principles of this invention, light from a light source member
 5 providing an extended, polarized light beam is directed through a transparent substrate
 and undergoes total internal reflection at the surface of the substrate by a single reflection
 within the TIR member. The reflected light is detected by a polarization-sensitive, two-
 dimensional array detector. The changes of the local polarization state in the beam's
 cross-section caused by the total internal reflection are employed to obtain information
 10 about the presence and composition in an array of substances on the substrate surface for
 each point of the surface. Total internal reflection is described in; M. Born, et al.,
 "Principles of Optics", 6th ed., pp 47-51, Pergamon Press, Oxford, 1991. In accordance
 with one aspect of the invention, the light generating element within the light source
 member is a quasi-monochromatic light source of moderate bandwidth. In a preferred
 15 embodiment, the light-generating element within the light source member is an LED of
 moderate bandwidth. The light from the light source member is directed through an
 internal reflection member to reflect off a specimen. The total internal reflection at any
 point within the cross-section of the light beam causes a phase shift between the light
 component polarized in the plane of incidence and the component polarized
 20 perpendicular to the plane of incidence. The reflected light is detected by a polarization-
 sensitive, two dimensional array detector and the signal from this detector is then
 processed in a computer to provide two-dimensional information about substances on the
 surface of the specimen. Spatially distributed changes in polarization state in the cross-
 section of the reflected beam are indicative of the substances in the specimen in the
 25 location in the specimen array corresponding to a position in the detector. The apparatus
 and method is especially adapted for imaging material in an aqueous solution. It is
 furthermore particularly suited for detecting attachment and detachment of analytes to a
 two-dimensional biomolecular array positioned on the total internal reflection member as
 part of a biosensor system. In various applications a plurality of discrete specimen spots
 30 are presented in an array, where the method and apparatus will image the array so as to
 distinguish each of the discrete specimen spots by an image which represents the change

in polarization state within each of the discrete specimen spots. Fluorescence or molecular tagging is not necessary nor practical for use in this invention.

Further, in accordance with the principles of this invention, the apparatus disclosed in the above-identified parent application provides an image of an entire array on a biochip or if desired a portion of the entire array.

The biochip slides used in accordance with the principles of this invention must have a roughness low enough to permit separate resolution of all of the spots on the array being imaged. The apparatus disclosed allows imaging at a level of greater precision such that, a lower degree of roughness on the surface of the biochip slide is required in order to make available the more precise information.

The image information comprises height and surface coverage information of the interaction which occurs, for example, between DNA or mRNA, the oligonucleotides deposited at each location in the biochip array and the constituent in the test material flowed over the biochip array. The height of such molecules as are found in bio-arrays can be as much as 300 Å-500 Å. Some biomolecules will have a height of lesser dimension. Consequently, the surface of the substrate on which the spots are located has to have a roughness of less than about 300Å in the measured area in order to avoid obscuration of the height information. Such precision is about equal to that of polished silicon wafers which cannot be used in the apparatus disclosed herein because silicon is not transparent. Roughness is a known unit of measure which is defined in Optical Society of America Handbook of Optics, Second Edition, volume 1, section 41.10.

Slides with such roughness characteristics used with the apparatus herein for biological testing are considered a significant departure from the prior art.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a block diagram of the invention.

Fig. 2 is a block diagram of an embodiment of the invention.

Fig. 3 is a block diagram of alternative portions of the invention.

Fig. 4 is a block diagram of alternative portions of the invention.

Fig. 5 is a block diagram of alternative portions of the invention.

Fig. 6 is a schematic projection view of a biochip useful in the apparatus of Fig. 1.

Fig. 7 is a schematic view of the slide of Fig. 2 showing a representative spot.

Fig. 8 is an hypothetical enlarged view of a biochip of the prior art representing the surface roughness of the slide and biomolecules; and

Fig. 9 is an enlarged schematic side view of a biochip slide having reduced roughness in accordance with the principles of the invention.

Fig. 10 is a schematic representation of a jig for holding the slide of Fig. 2.

Fig. 11 is a computer printout of an area plot of spots of a microarray.

DETAILED DESCRIPTION

The invention comprises a method and apparatus for analyzing a two-dimensional arrangement of chemical substances with an imaging technique. A polarized light source of known polarization state is directed into a total internal reflection member (TIR member) configured for a single reflection at a total internal reflection surface (TIR surface) and then exiting the TIR member. In the context of this document, superposition of reflections as encountered at a layered optical structure where the layer thicknesses are smaller than the coherence length of the illuminating light is referred to as a single reflection. The chemical specimen is in place above the TIR surface in the evanescent field of the reflected light beam. After reflection, the beam is passed to a polarization-sensitive two-dimensional detector such as a polarizer and a camera. The beam's content can then be processed to determine the change in polarization state, locally in the two-dimensional cross-section of the beam. This provides a spatially distributed map of change of polarization state in the specimen. A variety of techniques are available to determine the change in polarization such as measuring the deviation from a null condition or by comparing the input polarization state to the output polarization state.

The refractive index composition of the materials within the evanescent field determines the change in the polarization state of the beam due to the reflection at the TIR surface. A two-dimensional variation of this composition within the TIR surface is associated with a respective variation of the polarization state spatially distributed across the cross-section of the reflected light beam.

In one application, the chemical specimen forms a two-dimensional array of molecules (here referred to as receptors) with specific affinities towards respective other molecules

(here referred to as ligands). In this application, the invention is utilized to indicate the presence or absence of binding between ligands and receptors on the array. Such arrays commonly consist of a plurality of discrete specimen spots. The present method and apparatus will image the array so as to distinguish each of the discrete specimen spots represented by the local change in polarization state in the cross-section of the reflected beam.

Subject to limitations in resolving power of the detector, the invention permits measurement of thickness and/or refractive index composition of the specimen under investigation with a very high resolution, in the sub angstrom range, spatially resolved over an entire area. The invention is particularly useful in applications where the specimen is in an aqueous solution. In a particular application, the present invention is used to determine the presence of biological agents in a solution such as in immunosensor applications by measuring their attachment to antibodies on the TIR surface in the evanescent field. In another application, the present invention is used to determine the presence and structure of nucleic acid sequences in a solution by measuring their attachment to other nucleic acid sequences on the TIR surface in the evanescent field. Described in more detail below are different embodiments of the invention.

Referring to Figs. 1 and 2, an apparatus and method is illustrated which implements one embodiment of the invention. As shown in Fig. 1, the apparatus 10 can be described conveniently as consisting of three general portions. Portion 12 is a polarized light source assembly, portion 14 is a total internal reflection assembly providing a single reflection and portion 16 is a polarization-sensitive two-dimensional array detector assembly. Data from the detector assembly 16 is sent by an electrical signal 24 to processor 18 such as a specially programmed computer and user access system such as a print-out or image display. Data can be presented as an image, a data table, or in other forms. The polarized light source assembly 12 passes polarized light of known polarization state (which may be varied or varying) 20 to the total internal reflection assembly 14 where a single reflection occurs and the reflected light 22 having a changed polarization state passes to the detector assembly 16, where it is recorded spatially over the cross-section of the beam. The recorded data is sent to the processor 18 where the change of polarization state is determined to provide a spatially resolved map

of changes in polarization state. Where the specimens are presented as an array of discrete spots, each spot will be imaged for its change in polarization state within the spot area.

Fig. 2 shows a more detailed preferred embodiment. The polarized light source assembly 12 has a light source 26, a beam forming member 28 (if the nature of the light source is such as to make beam forming useful or necessary) a polarizer 30 and an optical retarder 32. The total internal light reflection assembly 14 has an optical element 34 which has an optical surface 36. Also shown is a specimen slide 38 on the optical surface 36, and between them an index matching substance 40. Because of the index matching a total internal reflection surface (TIR surface) is defined as the upper surface 39 of the specimen slide 38. A specimen 42 is on the total internal reflection surface 39 of the slide 38. The optical element 34 is a prism configured along with the index-matched slide 38 in relationship to the incoming light beam 20, and the exiting light beam 22 such that the beam reflects only a single time at the TIR surface 39 and then exits the prism. If the specimen is placed directly on the optical surface 36, then the optical surface 36 would be the TIR surface. But this is not the usual application as the specimen (such as a biochip) is usually prepared more conveniently on a specimen slide 38 and placed in the apparatus. In any event, however constructed, there is an optical structure having a TIR surface and the beam reflects only a single time at the TIR surface between entering and leaving the optical structure. In other words, there is a TIR surface in optical contact with the specimen, such that the evanescent field associated with the total internal reflection interacts with the specimen, and there is only a single reflection at that TIR surface.

The post reflection detector assembly 16 has a polarizer 44, and a two-dimensional array detector 46, preferably a camera of the CCD type. The processor 18 is a specially programmed computer and output means for processing the imagery into a representation of film thickness variations spatially resolved over the cross-section of the area imaged. The imaging is acquired by detecting changes spatially distributed in the local polarization state in the beam's cross-section caused by the total internal reflection. This provides information about the presence and composition in the array of substances on the substrate surface for each resolvable point on the surface. Different polarization state changes are included in the cross-section of the reflected beam indicative of the

substances on the specimen in the location in the specimen array corresponding to a position in the detector. The processor 18 receives the data as an electrical signal 24 and characterizes the change of polarization state spatially over the two-dimensional array. In the processor 18, the analysis and processing is done in one embodiment by comparing the known polarization state of the incoming light from the light processing assembly 12 with the changed polarization state of the reflected light 22, spatially resolved two-dimensionally within the beam which provides a map of spatially distributed points or spots in the specimen array. The polarization shift is then analyzed by the processor 18 to provide information of the presence and properties of elements in the chemical specimen. Other known techniques, such as null processing can be used to determine the change in polarization state.

Alternatively, the light source member 26 may be an LED, an SLD (Super Luminescent Diode), an incandescent light source, or a laser. If an LED or SLD is used, the set-up shown in Fig. 2 is appropriate, where the beam-forming member 28 is a collimator. If an incandescent light source is used, an optical filter is also used.

In one embodiment, the light source 26 for the apparatus is a quasi-monochromatic light source of moderate bandwidth. In accordance with the invention the light source 26 is preferably an LED of moderate bandwidth. Preferably the bandwidth is a full width half maximum wavelength in the range of about 10nm-50nm, and more preferably a full width half maximum wavelength in the range of about 30nm-50nm.

Referring to the optical retarder 32 as shown in Fig. 2, in an alternative embodiment, the optical retarder could be placed instead in the exiting beam path 22 before the polarizer 44.

Referring to Fig. 3, an alternative embodiment is shown. When the light source is a laser 50, a moving diffuser 52 is adapted to produce speckle-offsetting fluctuation of the minima and maxima in the speckle pattern caused by the laser. The moving diffuser 52 is attached to a mechanical actuator 54 which is preferably a motor and servo-apparatus for providing the speckle offsetting fluctuations. The beam 20 then proceeds through the beam-forming element 28, the polarizer 30 and the optical retarder 32, exiting the light source assembly 20.

The polarizer 30 employs a polarizer of selected known polarization state. The polarizer

30 may be of the type having a mechanical actuator driven by a motor control signal so as to enable varying and selecting the polarization state of the light beam 20.

As mentioned above, the total internal reflection optical element 34 either alone or in combination with an index matched slide may be arranged for use with a specimen in

5 various ways to define a total internal reflection assembly so long as the specimen is in the evanescent field of the reflected beam 20,22.

As noted above, the specimen 42 could be set directly on the optical surface 36 in which case the optical surface 36 would be the TIR surface but this is inconvenient and repeated use is likely to degrade the optical quality of the optical surface 36, and therefore,

10 consistent with common practice in which a biochip or other chemical assay specimen is provided, a specimen slide 38 or other supporting apparatus is employed. It is common in a biochip to provide an array of discrete specimen spots supported on a structure for obtaining analysis of each spot. The term total internal reflection optical element refers to known optical elements alone or in combination with other elements which provide the phenomenon known as total internal reflection. Fig. 2 shows use of a prism combined with a slide 38, being index matched so that there is a TIR surface 39.

Fig. 4 shows an alternative optical arrangement in which a flat optical member 56 having an upper surface 58 is surmounted by a specimen slide 60 and an index matching substance 62 on which is a specimen 64. The TIR surface 66 is the top of the slide 60.

20 The beam 20 enters the assembly, is refracted as it enters, and leaves the optical member 56 after a single reflection at the TIR surface 66 as beam 22. Other mechanisms for providing total internal reflection and an evanescent field can be employed in practicing this invention as long as only a single reflection occurs at the TIR surface upon which the specimen is placed so as to be in the evanescent field associated with the reflection.

25 As seen in Fig. 5, the post-reflection processing arrangement 16 through which the beam 22 passes, can alternatively, consist of a polarizer member 70, a beam forming member 72 and a two-dimensional array detector 74.

The method and apparatus can be used in combination with biochips of the type having discrete specimen spots or a micro-titer plate containing an array of discrete spots or

30 locations for analysis, where the detected change in polarization state is spatially related to the discrete locations in the reflected beam. Therefore, as used herein the slide and

specimen refers to any type of chemical or biological array which is desired to be examined.

The foregoing described apparatus and methods are especially beneficial for imaging materials in an aqueous medium.

5 The invention as described above provides an extremely sensitive optical imaging system for real-time imaging of the binding status of biochip array elements on the surface of a glass or plastic chip. An exemplary monitored array spot is approximately 25 μm in diameter, with a lateral resolution better than 10 microns, resulting in fully parallel, continuous real-time readout of up to 5 million sensor fields. Sensor sensitivity
10 to surface attachment is in the femtogram/ mm^2 range (e.g. one DNA per square micron) and spatial resolution of 20 microns.

The apparatus of Fig. 1 operates by imaging the pattern of reaction results on the biochip. Those reactions produce changes in the height of the resulting material which react at each spot, imaging an area of array containing numerous spots. The area imaged could
15 be a portion or the entire biochip array. By providing an array of spots of different materials, different constituents in test material flowed over the spots bind in a manner which identifies those constituents. By including in a computer memory the positions of the various materials in the different spots of the array, the image produced by the apparatus of Fig. 1 not only identifies the constituents in the test material, but also can
20 determine the rate at which the reactions occur and the height of the resulting molecules at each spot. With the apparatus described height differences can be imaged dynamically over such short periods of time that intermediate height change readings can be recorded and therefore height change rate can be determined as well as allowing comparison of the rate of height change or intermediate amount of height change among the for spots on the
25 biochip array.

In certain types of biochip analysis the height of molecules on the spots is on the order of 300Å to 500Å (Å = angstrom). Accordingly, the surface of a slide on which the spots are formed should have a maximum roughness of less than about 300Å in the measured area in order to avoid the loss of information in the resulting image. This is
30 particularly applicable to hybridization arrays of target nucleic acid and oligonucleotide arrays. For better imaging resolution, the slide roughness should be about 50% or less

than the height of molecules on the spots. Roughness is a known unit of measure which is defined in Optical Society of America, Handbook of Optics, Second Edition, Volume I, section 41.10.

In certain applications it is desirable that the surface roughness be less than the change in height caused by the binding reaction. Fig. 6 is a schematic projection view of a slide suitable for use in the apparatus of Fig. 1-5. The slide 110 has a top surface 112 on which an array of spots 114 is formed.

Fig. 7 is a side view of the slide of Fig. 6 illustrating a representative spot 116. Fig. 8 is an enlarged portion of a slide of Fig. 6 illustrating a plurality of spots after interaction in the context of an exaggerated surface roughness for the slide as in the prior art. The horizontal broken line 120 represents the maximum surface roughness as indicated by the opposing arrows 121 and 122. It can be seen from the figure that the height of a reacted spot at 123 is exaggerated as compared to a reacted spot at 124 and at 125. Similarly, unreacted spots at 126 and 127 distort the resulting image where they should provide an equal result.

Fig. 9 illustrates the point by showing the same interaction pattern where the surface roughness is less than a small portion of the height of the molecule produced by a reaction at each spot. Like numerals are used in Fig's. 8 and 9 to facilitate comparisons between the two figures.

In practice, the slide of Fig. 6 is positioned in a fixture 200 as shown in Fig. 10. The slide is inserted into the fixture by movement as indicated by the arrow 201, into a position represented by horizontal dashed lines 202 and 204. The slide is spaced apart from surface 36 of optical element 34 of Fig. 1. The spacing between surface 36 and the opposed bottom surface of the slide contains a fluid 206 of a matching index of refraction which, for all practical purposes, renders those surfaces invisible and providing for TIR only from the top surface 112 of the slide 10.

The biochip of Fig. 6 can include an array, for example, of 100,000 spots and those spots can be imaged simultaneously or in selected sub-arrays. Each spot has a diameter of from 50 microns to 500 microns where each spot comprises a biological element (i.e. oligonucleotides, cDNA clone, protein, antibody, antigen, bacteria, enzyme, inhibitor, receptor and others). Each spot has a known designation for, for example,

genes for breast cancer, prostate cancer, aging and others, - - - (up to 80,000 genes). The biological elements must be substances that can be attached by various chemical and physical techniques to create a biochip. A full discussion of biochip fabrication is included in "Microarray Biochip Technology" by Mark Schena, a Biotechniques Books
 5 Publication, 2000. "DNA Microarrays: A Practical Approach" by Mark Schena, a Practical Approach Series, 2000.

The binding status of biological array elements (spots) on the surface of a (glass) slide or chip is obtained by the apparatus of Fig. 1. The slide monitoring area is approximately 25 mm in diameter with a lateral resolution better than 10 microns,
 10 resulting in fully parallel, continuous, real-time readout of up to five million sensor fields. System sensitivity to surface attachment is in the femtogram/mm² range. The apparatus and chip have application to DNA and protein chip scanning, high throughput screening, ligand fishing, immunosensors, clinical diagnostics and research, toxicology profiling, binding kinetics research, genomics and proteomics. Particular features include; real-time
 15 imaging, no labels necessary, sensitivity of 1 fg/mm² (one DNA per square micron), partial resolution of 20 microns, and a sensor field of about 24 mm diameter.

The above described imaging apparatus was used to measure the thickness of protein layers deposited directly on the prism surface, which has a surface roughness of $\lambda/10$. Tests were performed by depositing small droplets (~0.5 mm in area, 200 nl in
 20 volume) of bovine serum albumin (BSA) solutions directly on the sensor surface (the TIR surface). After the water was evaporated, only the protein remained on the surface leaving a well-defined quantity of protein at the test location. Table 1 lists the number of molecules per drop for each concentration, as well as an estimate of the effective layer thickness based on the area the droplet covered on the surface. The intensities as
 25 measured by a CCD camera detector for the surface was computer plotted as an area plot and is shown in Fig. 11. From this figure, it can be seen that with an increase in the concentration of protein the signal increases. The plot readily distinguishes each spot. Very small measurements were detected. In particular, a thickness change of as little as 10 fm was detected and was distinguished from background noise. Consequently, with
 30 such sensitivity, in order to be able to exploit the increased precision measurement available from the apparatus, the surface roughness of the support element of the

microarray must be reduced.

Table 1. BSA dilutions and molecular quantities in sample droplets

Concentration	BSA mass per drop	BSA Molecules / drop	Moles per drop	Number of Molecular Layers	Effective Film thickness	Protein coverage
10 mg/ml	2 μ g	1.8×10^{13}	30 pmol	1400	10 μ m	4 μ g/mm ²
1 mg/ml	200 ng	1.8×10^{12}	3 pmol	140	1 μ m	400 ng/mm ²
100 μ g/ml	20 ng	1.8×10^{11}	300 fmol	14	100 nm	40 ng/mm ²
10 μ g/ml	2 ng	1.8×10^{10}	30 fmol	1.4	10 nm	4 ng/mm ²
1 μ g/ml	200 pg	1.8×10^9	3 fmol	0.14	1 nm	400 pg/mm ²
100 ng/ml	20 pg	1.8×10^8	300 amol	0.014	100 pm	40 pg/mm ²
10 ng/ml	2 pg	1.8×10^7	30 amol	1.4×10^{-3}	10 pm	4 pg/mm ²
1 ng/ml	200 fg	1.8×10^6	3 amol	1.4×10^{-4}	1 pm	400 fg/mm ²
100 pg/ml	20 fg	1.8×10^5	0.3 amol	1.4×10^{-5}	100 fm	40 fg/mm ²
10 pg/ml	2 fg	1.8×10^4	0.03 amol	1.4×10^{-6}	10 fm	4 fg/mm ²

In the broadest sense, one aspect of invention is in a biochip having a slide whose surface, which is the TIR surface, is of sufficiently low roughness specification that an array of spots imaged by the apparatus can be individually resolved using the apparatus described above.

A process using the apparatus and a biochip of such sufficiently low roughness is: placing the biochip surface in a flow cell in combination with the apparatus such that the surface of the slide which has the spots is the TIR surface; initially calibrating the apparatus such that light reflected from the biochip yields a fully-linear polarization;

adjusting the analyzer to a null position to fully block the linearly polarized light.

flowing target molecules over the biochip surface.

When the null position is achieved, every region where the chip (slide) surface deviates from the initial state stands out as a bright spot whose intensity is directly related to the thickness (height) differences induced by the deviation. In this process target molecules will be flowed over the biochip surface. The intensity of the biochip may be continuously monitored across the array to study thickness changes that occur on the biochip. The relative intensity measured at the detector will be related to the sample parameters and the setting of the polarizing elements using computer programs based on

a detailed "Jones calculus" sensor system description. With these programs theoretical plots will be fitted to the acquired measurement data sets, and the outputs' dependence on parameter variations can be visualized. At the start of the procedure, the entire biochip is homogeneously dark. As the antibodies begin to bind to the reference probes the

- 5 intensity along the surface increases. The intensity is expected to be the highest for high affinity interactions and mild changes are expected for low affinity interactions. The rate of intensity change can be related to the affinity constraints of the system. In a single procedure, the affinity measurements for multiple peptides can be performed. Moreover, the effect of binding density (because different concentrations of peptides are used in
- 10 spotting) can also be measured.

What has been described is merely illustrative of the invention and many variations and modifications thereof can be devised by those skilled in the art within the spirit and scope of the invention as encompassed by the following claims.